Effectiveness of Cross-Flow Microfiltration for Removal of Microorganisms Associated with Unpasteurized Liquid Egg White from Process Plant

S. MUKHOPADHYAY, P.M. TOMASULA, D. VAN HEKKEN, J.B. LUCHANSKY, J.E. CALL, AND A. PORTO-FETT

ABSTRACT: Thermal preservation is used by the egg industry to ensure the microbiological safety of liquid egg white (LEW); however, it does not eliminate all microorganisms and impairs some of the delicate functional properties of LEW. In this study, a pilot-scale cross-flow microfiltration (MF) process was designed to remove the natural microflora present in commercial LEW, obtained from a local egg-breaking plant, while maintaining the nutritional and functional properties of the LEW. LEW, containing approximately $10^{6\pm1.7}$ colony forming units (CFU) per milliliter of total aerobic bacteria, was microfiltered using a ceramic membrane with a nominal pore size of 1.4 μm, at a cross-flow velocity of 6 m/s. To facilitate MF, LEW was screened, homogenized, and then diluted (1:2, w/w) with distilled water containing 0.5% sodium chloride. Homogenized LEW was found to have a threefold lower viscosity than unhomogenized LEW. Influence of MF temperature (25 and 40 °C) and pH (6 and 9) on permeate flux, transmission of egg white nutrients across the membrane, and microbial removal efficiency were evaluated. The pH had a significantly greater influence on permeate flux than temperature. Permeate flux increased by almost 148% when pH of LEW was adjusted from pH 9 to pH 6 at 40 °C. Influence of temperature on permeate flux, at a constant pH, however, was found to be inconclusive. Microbial removal efficiency was at least 5 $\log_{10}$ CFU/mL. Total protein and SDS-PAGE analysis indicated that this MF process did not alter the protein composition of the permeate, compared to that of the feed LEW, and that the foaming properties of LEW were retained in the postfiltered samples.

Keywords: cross-flow MF, functional property, liquid egg white, microbial removal process technology

Introduction

Eggs and egg products are an important part of our food supply due to their high nutritional value and unique functional properties. A significantly increasing proportion of eggs are broken commercially for processing as liquid egg products (Ahn and others 1997). Liquid egg whites (LEWs), in particular, are essentially fat-free protein solution and contribute some unique functional properties such as foaming, coagulation, or emulsification to foods.

The interior of the avian egg is essentially sterile when freshly laid although eggs can be contaminated by pathogens such as *Salmonella*, via trans-ovarian transmission. Once broken, LEW requires pasteurization because improper washing, temperature abuse, and prolonged storage result in microbial invasion. Though invading organisms are approximately 60% Gram-positive, the primary spoilage organisms are Gram-negative. At refrigeration temperatures, the primary spoilage microorganisms are psychotrophs from the genera Pseudomonas and Aeromonas (MacKenzie and Skerman 1982).

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Because functional properties LEW can be easily impaired by heat treatment, pasteurization is conducted on a critical temperature–time regime where the egg protein coagulation is minimal. The USDA requires that LEW be heated at least to 56.6 °C and held for no less than 3.5 min (USDA 1969). However, despite adherence to the pasteurization protocols, outbreaks of foodborne illness from the consumption of egg products or foods manufactured with them as ingredients are still occurring. This is due to the fact that current pasteurization does not reduce microbial loading effectively from LEW and generally, only 1 or 2 log cycle reductions of viable cell counts are achieved by commercial thermal pasteurization, and pasteurized liquid egg products contain more than 10^2 or 10^3 microbial cells/g (Lee and others 2001). The principal genera found in pasteurized egg products are *Salmonella*, *Alcaligenes*, *Bacillus*, *Proteus*, *Escherichia*, *Pseudomonas*, and Gram-positive cocci (Schmidt-Lorenz 1983; Cunningham 1995). Hence, the shelf life of liquid eggs is usually short even under refrigerated condition.

Considerable effort has been devoted to overcome the weakness of conventional thermal pasteurization and to extend the storage stability of liquid egg using alternative technologies. However, most of these studies were focused on the microbiological point of view, and failed to consider the changes in physico-chemical properties of liquid eggs influenced by such processes. The alternative technologies reported for processing of liquid egg products include ultrasonic waves (Wrigley and Llorca 1992), irradiation (Ma and others 1993; Badr 2006), ultrapasteurization combined with aseptic packaging (Ball and others 1987),

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thermoradiation (Schaffner and others 1989), pulsed electric fields (Calderón-Miranda and others 1999; Fernandez-Diaz and others 2000; Amiali and others 2006), UV radiation (Unluturk and others 2008), and high hydrostatic pressure (Lee and others 2003, 2001, 1999; Andrassy and others 2006). However, microbial removal efficiencies of these processes were limited. Wrigley and Llorca (1992) reported Ultrasonic wave induced reduction in *Salmonella* in liquid egg ranged from 1 to 3 log CFU/mL at 50 °C whereas a irradiation dose of 3 kGy (Badr 2006) was found to be optimum for LEW reducing the total plate count by about 3.5 log. Ultrapasteurization (68.9 °C for 123 s) was found to provide about 3.2 log reduction in total plate count (Ball and others 1987). Pulsed electric field treatment is limited to small scale so far and the maximum log reduction using pulsed electric field and heat combined was reported to be 3.7 log CFU/mL for *Salmonella* in egg white (Amiali and others 2006). UV radiation has been reported to have produced greater than 2 log reduction for an *E. coli* strain in LEW (Unluturk and others 2008) whereas approximately 3 log reduction of *E. coli* in liquid egg was achieved by high hydrostatic pressure (250 to 400 MPa) at 5 °C (Lee and others 2001). Combinations of physical and chemical (antimicrobial) treatments are often necessary to overcome this process limitation.

MF is a membrane separation process (McGregor 1985; Zeman and Zydney 1996; Cheryan 1998), which has been shown to removes large cellular components and contaminants from a fluid feed such as milk while proteins, water, and other smaller solutes pass through the membrane. In cross-flow filtration, the feed flow is parallel to the filtration medium and permeates through the membrane due to a pressure difference. Thus in MF, selection of membrane of appropriate size is capable of excluding both vegetative cells and spores entirely from a liquid feed rapidly with minimal loss of nutrients and functionality, which represents a major advantage over conventional thermal processing. However, removal of microorganisms from fluid food using MF may not be solely due to size exclusion but also to factors, such as affinity between the ceramic membrane and cellular structure of organism and the intensity of shear forces on the microbes during the process (Kang and others 2004). Microbial cells are affected and damaged by fluid hydrodynamic forces, which are produced during MF in both laminar and turbulent situation. Cells vary a great deal in susceptibility to shear depending on cell type and growth stage. The shear response to cell is determined by the intensity, duration, and type of the force. Turbulent shear stress usually cause more damage than laminar shear stress of the same magnitude (Chisti 2001).

Permeate flux, which is defined as the volume of the permeate per unit cross sectional of the membrane per second, is a measure of the performance of cross-flow MF and depends largely on feed concentration, temperature, and feed-flow rate. Permeate flux increase with transmembrane pressure, which is the differential pressure between the feed side and permeate side, until a maximum flux or critical flux is achieved. A further increase in transmembrane pressure results in decrease in permeate flux. With decreasing transmembrane pressure, the flux increases again but often to a lower value than during the pressure-increasing mode. This is due to the fact that increase in transmembrane pressure above critical flux led to the formation of fouling on the membrane surface. It is, therefore, important to increase the transmembrane pressure stepwise to obtain optimum or critical flux region (Bacchin and others 2006). As time progress, the permeate flux declines mainly due to membrane fouling and other factors such as the morphology of the membrane together with fluid mechanics attributed to the flow of the feed across the membrane (Van Der Horst and Hanemaaijer 1990).

For application and optimization of MF processing in a real food system like LEW or liquid whole egg, information on operating parameters, effectiveness of microbial removal efficiency, and the influence of the process on egg white protein functionality are important. The literature on MF of LEW as a microbial intervention technology is scarce. Ferreira and others (1999) reported MF of depth-filtrated ovomucin-depleted egg white in a bactocatch unit. However, the researchers reported a low reduction of microbial load. The microbial removal efficiency of the MF process was 1 to 2 logs only.

The objective of this study was to design a process using a tangential cross-flow MF to remove all types of microorganisms present in commercial unpasteurized LEW and to examine the influence of process parameters on the functional properties of the LEW.

Materials and Methods

Liquid egg white

For the present study, frozen unpasteurized LEW was obtained from Michael Foods (Klingerstown, Pa., U.S.A.). Authorization for purchase of the LEW was granted under section 590.10 by District Manager, USDA, FSIS/OFO (Philadelphia, Pa., U.S.A.). Chalaza, pieces of broken shells, and other undesired materials were removed from LEW at the breaking plant by wedge wire screening. Breaking plant utilized a 316 Stainless Steel, 0.030 wedgewire insert (25 wire mesh equivalent –750 micron) with a processing capacity of approximately 45000 pounds of egg whites every 4 h. Chalaza is the ropey strands of egg white which anchor the yolk in place in the center of the thick white. Upon shipment, the LEW was kept frozen at −20 °C until use.

Viscosity

Viscosity was measured in a Brookfield programmable rheometer (Model DV III ultra, Brookfield Engineering Labs, Stoughton, Mass., U.S.A.) using spindle nr LV2 and Rheocal software (version 3.1-1). For each measurement, about 75 mL of LEW test sample were used and temperature was maintained at 25 °C.

Homogenization

The frozen LEW was removed from the freezer and kept in a 4 °C refrigerator (approximately 48 h) to thaw the material completely. This thawed LEW was homogenized in a Universal Pilot Plant (Processing Machinery & Supply Co., Philadelphia, Pa., U.S.A.) in 2 stages (2500 and 500 psi) at 6 °C. Homogenization was introduced to bring homogeneity and lower the viscosity of LEW to facilitate MF. The fluidity of homogenized LEW was noticeably improved.

Feed LEW

The wedge wire screened and homogenized commercial LEW was found unsuitable for MF for removal of microorganisms due to the blockage of the membrane. To improve the feasibility of this process, the homogenized LEW was diluted and this homogenized and diluted LEW was then successfully microfiltered. In this study, the homogenized LEW was diluted with deionized water (1:2, w/w) containing 0.085 M food grade sodium chloride (CAS nr 7647-14-5, Mallinckrodt Chemicals, Phillipsburg, N.J., U.S.A.). The pH of the LEW was adjusted to 9 and 6 for experimental runs using either 6 N HCl (CAS nr 7647-01-0, Mallinckrodt Chemicals) or 6 N NaOH (CAS No. 1310-73-2, Fisher Scientific Co., Rochester, N.Y., U.S.A.). This homogenized, diluted, and pH adjusted LEW was kept in a

refrigerator overnight, at 4 °C, prior to microfiltration and the clear LEW from the top was used as feed in MF experiments.

Microfiltration system

Experimental runs were conducted using a Membralox Pilot Skid System (Pall Advanced Separation System, Cortland, N.Y., U.S.A.), which was designed for testing the feasibility of microfiltration to separate particulate contaminants from a liquid food by passage through microporous membrane. A 19-channel ceramic double layer, Membralox GP membrane (part nr S700-01246) of 4-mm channel diameter and 1.02-m channel length was used in all runs. The membrane support (12 microns), membrane layer (1.4 microns), and the end sealing all were made of pure alpha alumina. The average pore size of the membrane was 1.4 micron. GP membrane provides optimum soluble macromolecules transfer across the microfiltration membrane. In conventional microfiltration conditions, the natural pressure drop creates an asymmetric transmembrane pressure (TMP) from the inlet to the outlet of the flow channel. TMP is the differential pressure between feed side and permeate side and is calculated by subtracting the permeate pressure from the average of feed and retentate pressures. To correct this TMP decrease, GP membranes have a longitudinal permeability gradient built into the support structure without modification of the filtration layer. This design ensures a stable microfiltration regime, which provides a constant TMP all along the membrane.

A schematic diagram of the MF process is shown in Figure 1. MF housing is manufactured to sanitary 3A standard. The pilot unit contains a feed vessel of 30 gallon capacity, a feed recirculating pump, membrane module containing the ceramic membrane, a heat exchanger unit to heat the feed, a back pulse device to prevent membrane fouling, pressure gauges to detect pressure inside feed, permeate and retentate lines, flow meters for feed, permeate and retentate, and a number of ball valves at various collection streams.

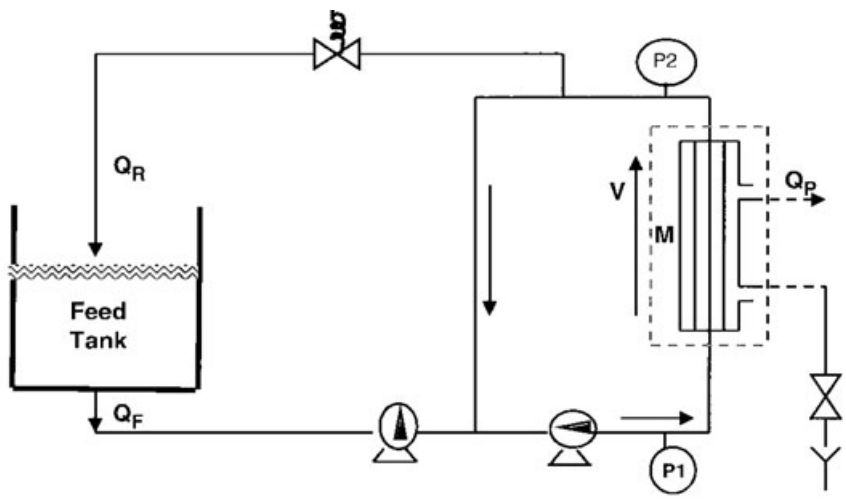
In tangential cross-flow configuration, the feed LEW is pumped tangentially over the ceramic filter membrane and filtered clean LEW flows out through the shell side or the perimeter of the membrane as what is known as permeate.

Microfiltration experiments

During the experimental runs, the vessel was charged with approximately 25 gallons (approximately 80% of capacity) of diluted and pH adjusted feed LEW and was heated up from 4 °C to the experimental temperature by heat exchanger unit. Experiments were carried at 2 different temperatures, namely 25 and 40 °C, and at each experimental temperature, the MF performance was investigated at 2 different pH, namely pH 6 and pH 9. Temperatures above 40 °C were not considered due to high heat sensitive nature of egg white proteins; although a better fluidity could be achieved at a high temperature. The feed temperature was accurately controlled with in ±1 °C and feed LEW was equilibrated at the experimental temperature for approximately 25 min to stabilize the contents of feed LEW. The pump speed was then adjusted to 65% of the maximum cross-flow velocity and the contents of the feed tank were pumped to the module supporting the ceramic microfilter using the recirculation pump. During experiments, backpulsing was used only when there was an appreciable decrease in permeate flow. Back pulse is a device that reverses the filtrate flow back into the module to lift the foul layer from the membrane.

MF process aims to remove microorganisms from unpasteurized feed LEW. However, the permeate stream from the MF process expected to contain water and of salt (0.005 g/mL). The egg white protein in the permeate can be concentrated by removing water. Ultrafiltration has been used to concentrate egg white proteins by partially removing water and other low molecular weight species. Froning and others (1987) reported concentrating the dilute egg white from 11.4% to 23.24% while lowering salt levels by almost 50% as compared to initial level. Another way of achieving the objectives of egg white protein concentration and salt removal is to use ultrafiltration combined with diafiltration, where water is added to the retentate stream at a rate that exactly balances the permeate flow (Jaffrin and Charrier 1994).

Clean water permeability (CWP) of the membrane was determined each time prior to start of an experiment. For this, the feed tank was first filled with deionized (DI) water at experimental temperature and the pump speed was set to 65%. Then water was recycled through the membrane with the permeate valve closed for 5 min. The water was prefiltered with a 0.2 μm filter. The pump



M- Membrane module; Q_F-Feed Liquid Egg White; Qp-Pathogen Free Liquid Egg White as Permeate; Q_R-Retentate; V- Cross Flow Velocity; P1, P2-Pressure Gauge

Figure 1 — Schematic diagram of USDA membralox pilot system (Pall Corp.) used for LEW microfiltration.

was then stopped and the water was drained from the feed tank. The feed tank was then refilled with deionized water and the pump restarted. The permeate valve was then opened to rinse the shell side of the membrane. The permeate flow rate was measured as a function of time. CWP is the volumetric flow of clean water through area of membrane surface per hour and is typically expressed in liter per square meter per hour per unit transmembrane pressure. If the measured CWP was significantly lower than the expected value, the standard cleaning procedure for membrane must be repeated. The CWP value should be within 10% to 15% of that measured at the first clean water permeability test under the same conditions to confirm cleaning validation.

Membrane cleaning

Although cross-flow filtration minimizes the build-up of foulants on the membrane surface, regular chemical cleaning is required to ensure optimum performance and long membrane life. The rate of heating and cooling should not exceed 10 °C per minute during cleaning process and the water used for cleaning must be softened or deionizied.

Usually, at end of each run, the system was drained and filled with water. Permeate outlets were closed so that the TMP is negligible. This ensures foulant removal by the cross-flow without redeposition on or in the membrane. The system was then flushed to drain with water at same temperature until the retentate stream appears clear. Then system was recycled with a cleaning solution containing 1% (w/w) caustic soda (NaOH) and bleach (400 ppm of free chlorine) for 15 min at 50 °C. This precleaning removes dirt in the system piping and weakens the deposit surface layer. Permeate valve remains closed during this step. Retentate side was drained and Recycle with caustic soda solution at 60 to 80 °C for 30 min with permeate valve closed. Then the permeate valve was open slowly and rinsing was continued for another 30 min. This ensures that the membrane, the ceramic support, and the permeate side are cleaned. Both retentate and permeate sides of the module was then drained. The system was flushed with water at the same temperature until the pH is close to neutral. Permeate CWP was checked. The CWP value should be within 10% to 15% of that measured at the first clean water permeability test under the same conditions. In case of a lower CWP compared to target, additional cleaning with nitric acid (HNO3) was followed. For this cleaning step, the permeate valves were closed and 1% (w/w) nitric acid was circulated at 60 to 70 °C for 15 min. This solubilizes precipitated inorganic residual salts. Permeate valves were then opened slowly and cleaning was continued for an additional 10 to 20 min. Both retentate and permeate sides of the module were then drained and the system was flushed with water at same temperature until the pH was close to neutral. The CWP was checked again and compared with values for new membranes to confirm cleaning validation.

Microbiological analysis

Both permeate and retentate samples were withdrawn aseptically at regular intervals during experiments. The total aerobic bacteria and yeasts and molds (YM), lactic acid bacteria (LAB), coliforms, and Salmonella spp. levels were enumerated in LEW. Total aerobic bacteria and YM were enumerated by serial diluting the LEW sample in 0.1% sterile peptone water as needed and spread plating 100 or 250 μL onto duplicate Plate Count agar (PCA; Difco, Becton Dickinson, Sparks, Md., U.S.A.) plates or potato dextrose agar (PDA; Difco), respectively, and incubating for 72 h at 30 °C. For enumeration of total LAB, the LEW sample in 0.1% sterile peptone water was serial diluted as needed and spread plated 100 or 250 μL onto duplicate Mann, Rogosa, and Sharp (MRS; Difco) agar plates and incubated anaerobically (10.1% carbon dioxide, 4.38% hydrogen, and balance nitrogen; Bactron IV Anaerobic/Environmental Chamber, Sheldon Manufacturing Inc., Cornelius, Oreg., U.S.A.) for 48 h at 37 °C. The coliforms levels were determined by serial diluting the LEW sample in 0.1% sterile peptone water as needed and spread plating 100 or 250 μL onto duplicate MacConkey (Difco) agar and incubating for 48 h at 37 °C. Salmonella spp. were enumerated by serial diluting the LEW sample in 0.1% sterile peptone water as needed, spread plating 100 or 250 μL onto duplicate xylose-lysine-tergitol-4 (XLT4; Difco) agar, and incubating for 48 h at 37 °C. Bacterial numbers were expressed as $\log_{10}$ CFU/mL.

Foaming property

The foaming property of LEW samples (feed, permeate, or retentate) was measured and expressed as foaming power and foam stability (Phillips and others 1990). The LEW foam was prepared by beating LEW using Sunbeam Mixmaster (Model 2350, Boca Raton, Fla., U.S.A.) equipped with double whipping beater. The pH of LEW sample was first adjusted to 7. A total of 150 mL of LEWs were placed inside the bowl of the mixer and then beaten for 10 min at the speed setting of 10, which was specified in the mixer as the "egg whipping speed." The foam produced were gently and carefully filled into 3 tared weighing boats (100 mL) using small scoops and avoiding entrapped air pockets. Excess foam was scraped off from the top of the boat using a metal spatula to level the top of the foam even with the top of the weighing boat to obtain constant volume for each measurement. The foaming power was expressed in terms of % Overrun (OR).

$$\%OR = ((\text{Wt . of 100 mL LEW/Wt. of 100 mL of foam}) - 1) \times 100$$

Foam stability (g, drained foam) was determined by monitoring the drainage of foam through a small hole (approximately 0.6 cm) in the bowl. The drained liquid was collected in a tared container on the balance pan and the weight of drained liquid after 1 h was recorded as the foam stability. Foaming property was measured in triplicate for each experimental sample.

Total solids

Total solids (TS) was determined according to AOAC official method 925.31 for egg and egg products (AOAC 1995). Two grams of the LEW sample was weighed in duplicate in aluminum dish and dried in the Fisher Isotemp oven (Model 230 F, Fisher Scientific, Pittsburgh, Pa., U.S.A.) at 100 °C for 1 h 15 min according to the procedure. Samples were then placed in a desiccator, weighed, and the difference in weight was recorded as moisture content (%TS = 100 – moisture%).

Total protein

Nitrogen content was determined according to AOAC official method 925.31 on duplicate LEW samples using an FP-2000 nitrogen analyzer (LECO Corp., St. Joseph, Mich., U.S.A.) with the combustion chamber set at 1050 °C. Total protein (TP) was calculated from total nitrogen content by multiplying by 6.25.

SDS-PAGE

SDS-PAGE analysis based on the procedure by Laemmeli (1970), was modified for use on the PhastSystem® (GE Healthcare, Piscataway, N.J., U.S.A.). Liquid samples (final concentration of 1 to 2 mg protein/mL) were dispersed in 0.08 M Tris, 0.0005 M EDTA, 3.5% SDS, pH 6.4 containing 10% mercaptoethanol, and 0.025% bromophenol blue. Low molecular weight markers (94 to 14.4 kDa; GE Healthcare) of known molecular weights were used to help

identify protein bands. Electrophoresis was conducted on a Phast-Stystem using ultra-thin precast 12.5% acrylamide gels with SDS buffer strips. Gels were stained with 0.1% Coomassie Brilliant Blue and destained in water/methanol/acetic acid (6/3/1, v/v/v). Gels were scanned into a densitometer (375A Personal Densitometer SI equipped with Molecular Dynamics, Sunnyvale, Calif., U.S.A.) and protein profiles analyzed using Image Quant software (version 4.2, Molecular Dynamics).

Data analysis

Permeate flux was calculated from the volumetric flow data of permeate through per unit area of membrane surface per hour using a spreadsheet (Microsoft Excel, Microsoft Corp., Redmond, Wash., U.S.A.). The experiments were duplicated and the means of 2 sets of data are reported in this article.

For bacterial population count, duplicate samples were taken each time during an experiment and the average was calculated, multiplied by the dilution factor and expressed as natural log number of survivors. For the evaluation of foaming ability and foam stability, experimental samples were analyzed in triplicate. The unpaired *t*-test was used to evaluate the significance of the pathogen removal and foaming properties. Foaming stability data were analyzed by one-way analysis of variance (ANOVA) using GraphPad software (La Jolla, Calif., U.S.A.). In all cases, significant difference was based on the 5% level ($P \leq 0.05$).

Results and Discussion

Viscosity

During MF experiments, samples were withdrawn at various stages of operation and the viscosity was measured. It is evident from Figure 2, that homogenized "feed LEW" has almost three-fold lower viscosity than industrial LEW. The reduced viscosity and associated improved fluidity is one of the important factors that made MF of LEW feasible. During homogenization, pressure induced phenomena such as cavitation, shear, and turbulence are involved simultaneously. Collapse of cavitation bubbles induces high-pressure gradients and high local velocities of liquid layers in their vicinity. These in turn cause shear forces, which are capable of breaking down protein molecules and alter the fluid viscosity (Dumay and others 1996).

Although protein structure is susceptible to modification by homogenization, it is not known to what extent it influences functionality of LEW. The water dilution provided additional fluidity suitable for the passage of LEW across the porous membrane. Salts have been reported to affect electrostatic interactions in macromolecules by contributing to the ionic strength. The lyotropic effect of salt is a linear function of its concentration up to 0.15 M (Srinivasan and Kinsella 1982). Addition of sodium chloride (0.085M), in this study, improved the stability of the soluble protein matrix in the mixture probably due to salting-in effect (Kakalis and Regenstein 1986).

Influence of cross-flow velocity

A proper cross-flow velocity needs to be maintained during MF since it influences the formation of fouling layer on the membrane surface and hence the flux (Zeman and Zydney 1996). Fouling involves the combined effects of physical, chemical, and biological factors (Nagaoka and others 1996; Chang and Lee 1998). The influence of cross-flow velocity on LEW MF is shown in Figure 3. Fouling during MF of LEW is mainly due to concentration polarization and can be controlled by proper choice of operating parameters, such as shear force induced by the cross-flow velocity. Permeate flux was found to increase by approximately 20% as cross-flow velocity increased from 4.8 to 6.2 m/s probably due to removal of retained particles and hence reduced the boundary layer thickness at higher cross-flow velocity during MF of LEW. All MF experiments with LEW were conducted at a cross-flow velocity of about 6 m/s.

Although a higher permeate flux is achievable at a much higher cross-flow velocity, this option was not pursued since use of higher cross-flow velocities leads to high energy consumption. Cross-flow velocity is also a function of TMP along the filter. The change in permeate flux with TMP at different cross-flow velocity during MF of LEW is given in Figure 4. By increasing the cross-flow velocity from 6 to 8 m/s the permeate flux could be increased by

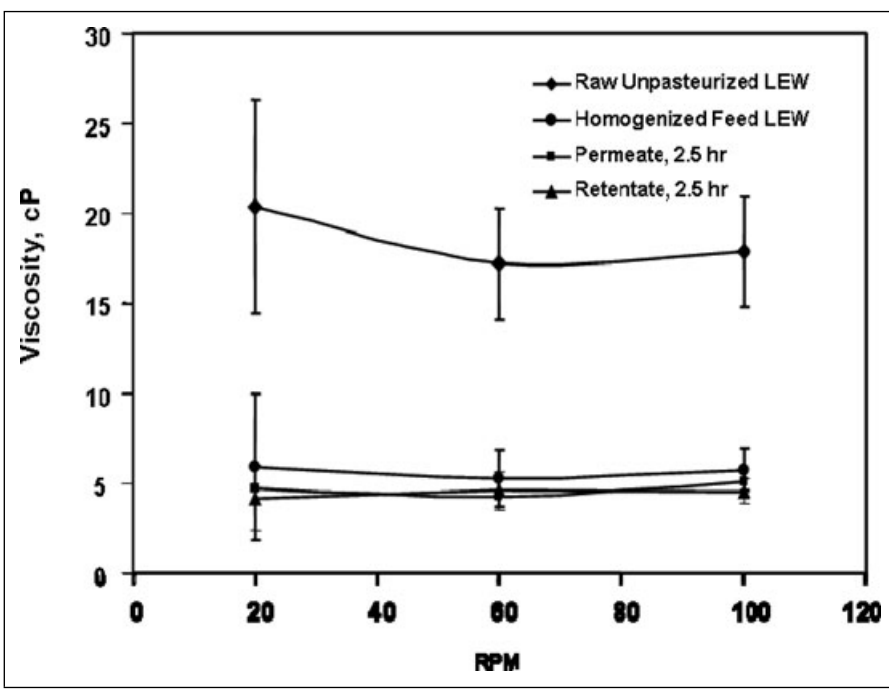


Figure 2—Viscosity of raw, homogenized, and microfiltered (permeate and retentate) LEW. Error bars represent standard deviations.

approximately 33% (Figure 4). However, this would require 77% increase in energy consumption since energy consumption increases proportionately to the square of cross-flow velocity. In this study, a cross-flow velocity of about 6 m/s was used. This choice reduced the energy consumption substantially by operating at low TMP while at the same time provided reasonably good permeate flow. Low TMP operation is possibly a wiser choice considering the possibility of shear damage to delicate egg white protein and to the ceramic membrane.

Influence of temperature and pH

The profiles of permeate flux as a function of pH and temperature is shown in Figure 5. We observed during MF of feed LEW, there is a large drop in permeate flux initially and although the flux continued to decrease during the entire experiment, the rate of this drop, after initial equilibration, was much smaller compared to initial drop. Feed temperature and the physical–chemical properties of feed such as pH, have impact on permeate flow through the membrane and hence permeate flux (Figure 5). The decrease in permeate flux is probably due to fouling and compaction of boundary layer. At 40 °C, the average permeate flux was found to increase from approximately 39 L/h.m^2 for the run at pH 9 to approximately 98 L/h.m^2 for the run at pH 6. This is amounts to about 148% increase in permeate flux. Similar increasing trend of flux with LEW pH, although much lower (22%), was observed at 25 °C. The influence of pH on permeate flux, is therefore, quite significant at 40 °C. However, the influence of temperature on permeate flux at a constant feed pH was found be inconclusive. At pH 6, the flux was increased by 36% as the temperature increased from 25 to 40 °C. However, at pH 9, the trend was reversed. Ferreira and others (1999) conducted MF of egg white depleted in ovomucin in a bactocatch installation under similar operating temperature and pH. A depth filtration step was performed in the study prior to MF, which affects the feed composition. Like the present study, the researchers reported maximum permeate flux at pH 6 and 40 °C. However, no direct comparison could be made since the authors did not report the permeate flux profile. Bolay and Ricard (1995) reported MF of crude dried egg albumin solution in water at 25 °C and at pH ranging from 3.7 to 7.5 and observed a strong dependence of permeate flux on pH. The researchers observed maximum flux at pH 5.2 due to neutral overall charges of membrane/protein system (zero streaming potential). This finding also in agreement with the present study with LEW where maximum flux obtained at pH 6. Membrane fouling during microfiltration of protein solution may result from several phenomena including electrostatic adsorption, hydrophobic interaction, hydrogen bond interaction and asymmetric pore blockage. The extent of these phenomena depends, among other factors, on the solution pH. At pH 6, which is close to protein isoelectric pH, the overall charges of the protein-membrane system are neutral. The protein being positively charged, adsorbs and progressively neutralizes the membrane, which is negatively charged, causing an increase in flux. The optimum pH with regard

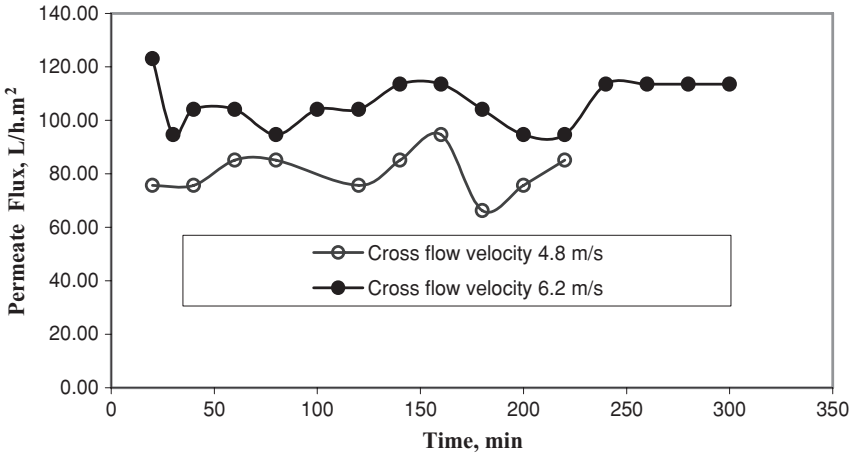


Figure 3—Influence of 2 different cross-flow velocity on permeate flux for 1.4 micron ceramic membrane.

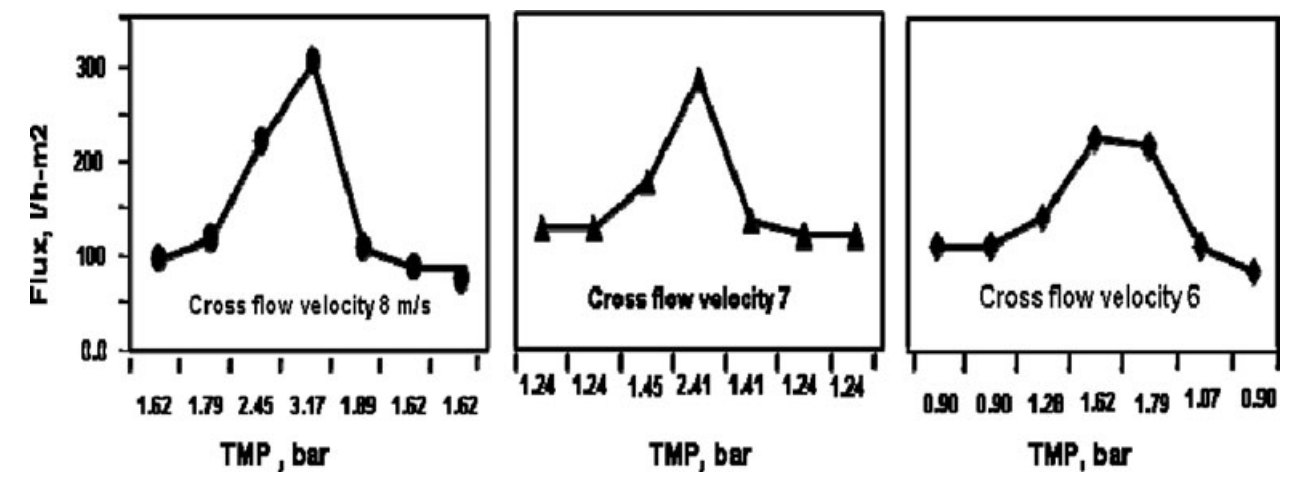


Figure 4—Permeate flux against TMP at different cross-flow velocity.

to flux decrease is one where the component in the solution is highly charged, and preferably with the same sign of charge as the membrane surface. At pH 9, the charges on the egg white protein molecules are negative and molecules are stretched due to electrostatic repulsion. In this situation, adsorbtion via hydrophobic interaction and/or hydrogen bond creates increasing amount of negative charge in the protein-membrane system with time. At the end of adsorption process, pore blockage continues and as a result permeate flux declines.

Microbial intervention

The level of contamination in egg shell and hence in liquid egg in the breaking plant varies widely and the population may ranges from 4.3 to 7.7 log CFU/mL, and bacteria from 16 genera were found in a survey (Board and Tranter 1995). The microbiological results of MF intervention processes are shown in Figure 6. Due to the presence of different types of organisms, LEW samples were analyzed for 4 major groups: total aerobic bacteria, LAB, yeasts and molds (YM), coliforms, and *Salmonella*. Figure 6 represents overall log reductions of various types of microorganisms as indicated. We did not detect the presence ($\leq$0.25 CFU/mL) of any Salmonella species in the in-coming raw unpasteurized LEW from the plant. Due to the wide variation of microbial populations (4.3 to 7.7 log CFU/mL) in the raw LEW supply, the average result of all 8 runs was considered. The experiment design was 2 $\times$ 2, based on 2 levels of operating pH (6 and 9) and temperature (25 and 40 °C). During each of the 8 experiments (2 pH levels $\times$ 2 temperatures $\times$ 2 trials), duplicate samples were withdrawn at multiple times, usually at 0, 5, 10, 30, 60, 150, and 240 min into the experiments. We observed the

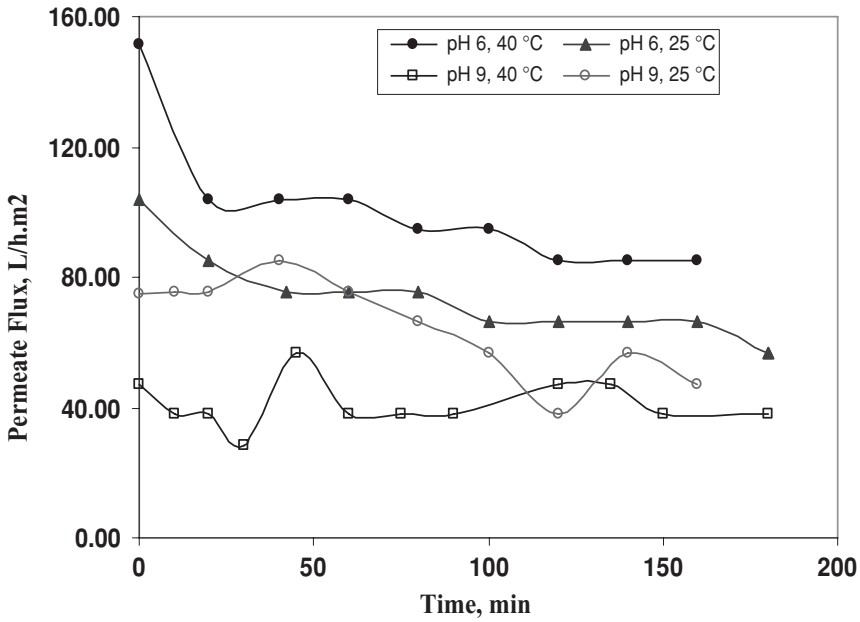


Figure 5—Permeate flux profile of LEW microfiltration under various operating conditions.

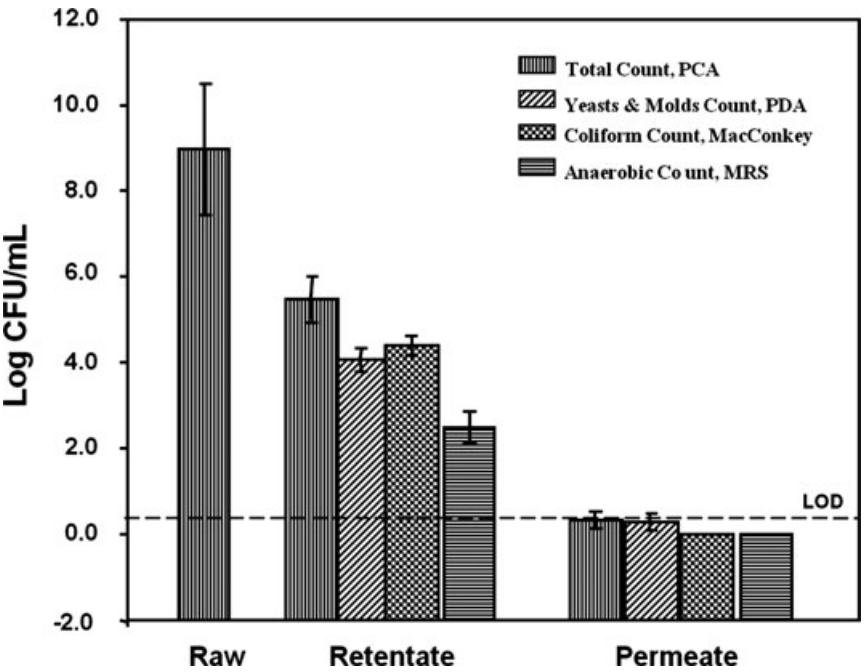


Figure 6—Effectiveness of pilot scale microfiltration for removal of microorganisms from industrial unpasteurized LEW. Error bars represent standard deviations. LOD is the limit of detection.

absence (≤0.25 CFU/mL) of any of the target microorganisms in the permeate stream (product) within the first 10 min. Out of the 8 experiments, in 2 experiments at pH 6 and at 25 °C, the total aerobic count and the YM count in the permeate were 0.33 and 0.29 CFU/mL, respectively. Irrespective of experimental conditions, MF process successfully removed all incoming microorganisms from the feed LEW (Figure 6) with in 10 min. We did not detect (≤0.25 CFU/mL) any microorganism in the product LEW (permeate) from MF.

Foaming properties

Egg white has many useful functional properties and one of such property is foam formation. Foaming is the incorporation of air into a food matrix, usually by whipping. "Foaming power" of LEW before and after MF is shown in Table 1. The foaming power of LEW is attributed to the egg white proteins, which produce a rigid membrane structure surrounding the entrapped air bubbles.

The weight of drip liquid from the foam upon standing is given in Table 1 as a measure of "foam stability." The table provides the information regarding the average foaming property of the 8 experimental samples. Each experimental sample was analyzed in triplicate for the evaluation of foaming power and foam stability. On average, the permeate LEW was found to have 17% higher ($P > 0.05$) foaming power than feed counterpart where as the retentate foaming power was basically the same as the feed. With regard to foaming stability, test samples before (feed) and after microfiltration (permeate and retentate), on average, have similar foaming stability with less than 5% variation. Statistical analysis indicates that there is no significant difference in foaming power and foam stability among feed LEW, permeate, and the retentate. Therefore, MF did not affect the foaming properties, namely foaming power and foam stability, of feed LEW.

Table 1—Foaming power and foam stability of LEW samples before and after microfiltration process.

Sample	Average foaming power, %*OR*	Average foam stability, gram of drained foam
Homogenized, treated feed egg white before microfiltration	640.8 ± 202.3	119.5 ± 4.26
Permeate after microfiltration	748.9 ± 260.0	114.3 ± 10.5
Retentate after microfiltration	634.8 ± 266.7	119.4 ± 4.2

Total solids and total protein

Total solid and total protein content of permeate and retentate samples were analyzed and compared with the feed LEW used for microfiltration. Result of analysis of TS and TP in various LEW samples during experiment have shown in Figure 7, which provides the information regarding the average TS and TP of experimental samples. The experimental samples were analyzed in duplicate for TS and TP. On average, the percentage of TP in the feed and permeate were 2.7 ± 0.33 and 2.4 ± 0.5, respectively, where as that of TS in the feed and permeate were 3.92 ± 0.63 and 3.97 ± 0.22, respectively. It is evident that (Figure 7) the amount of TS and TP of feed LEW did not change appreciably before and after MF.

SDS-PAGE analysis

Protein profiles of the different LEW preparations were very similar with prominent ovalbumin (45 kDa), ovatransferrin (77 kDa), and lysoszyme (14 kDa), and minor ovamucoid (28 kDa) bands. Protein distribution in the unhomogenized and homogenized LEW samples were similar and contained 55% to 60% ovalbumin, 9% to 13% ovatransferrin, 11% ovamucoid, and 8% to 10% lysozyme. This is similar to the distribution of egg proteins reported by Desert and others (2001) except for lysozyme.

Egg whites typically contain approximately 3.5% lysozyme. The higher amounts seen in our gels were elevated because it was difficult to separate it from the staining associated with leading edge. Also, we did not detect ovomucin in our gels, probably due to its removal during screening of LEW in plant operation. The protein profiles for the feed LEW and the resulting permeates were essentially the same although 2 different pHs treatments (pH 6 and pH 9) were performed on these samples. The microfiltration of commercial, homogenized, diluted egg whites did not alter the amount of the 3 major proteins, ovalbumin, ovatransferrin, and ovaglobulin.

Conclusions

Microfiltration as a unit operation intervention technology for removal of egg borne microorganisms present in unpasteurized LEW from industrial egg breaking plant has been described. The pilot scale process is capable of removing all groups of microorganisms from the feed LEW. In the process, raw LEW was homogenized and diluted with 2 times the amount of water for successful

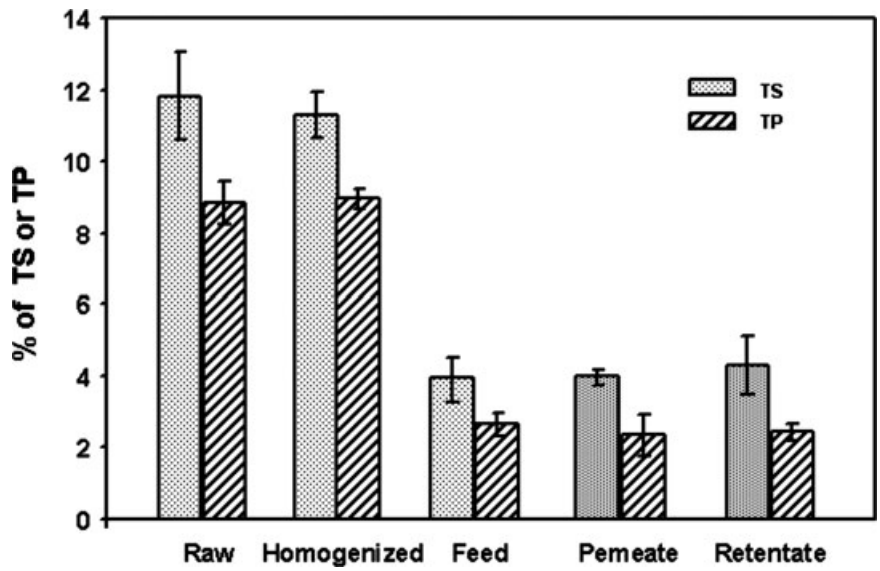


Figure 7—Estimates of total solids (TS) and total protein (TP) of microfiltered and control samples. Error bars represent standard deviations.

membrane separation. The homogenized feed had almost three-fold reduced viscosity. A cross-flow velocity of about 6 m/s was found to be most suitable. The operating parameters was found to have large impact on the permeate flux. Influence of pH was greater than the influence of temperature. Foaming properties, namely foaming power and foam stability, of feed LEW were found to be unaltered by the MF process. TP and TS contents of feed were also remained unchanged during MF. SDS-PAGE and densitometer analysis reveals that the microfiltration of "feed LEW" did not alter the distribution of proteins and that the amount of the major proteins remained same after microfiltration. The permeate from the process is essentially microorganisms free and yet retains the functional properties of the feed LEW. This pathogen free permeate is well suited for dehydration by spray drying to make powdered egg white. To avoid problems related to circulation due to high viscosity, liquid eggs are usually diluted with water, preferably in 1:2 ratio (Ayadi and others 2008). The pathogen free LEW from the cross-flow microfiltration process thus serve as an ideal starting material for making dry egg white powder by spray drying. Dried egg white has a number of advantages over LEW and is usually preferred in the confectionery industry. Alternatively, the microorganism-free dilute LEW from the microfiltration process may be ultrafiltered to remove salt and water. Ultrafiltration has been used to concentrate proteins in liquid foods such as LEW and to remove sugar and salt (Froning and others 1987).

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